

REMARKS

In the Office Action dated October 28, 2004, Claims 1, 3-6, 14-17 and 38 are pending. Claims 4 and 15 have been withdrawn from consideration as drawn to non-elected subject matter. Therefore, Claims 1, 3, 5-6, 14, 16-17 and 38 are currently under consideration on the merits.

In the first instance, Applicants, through the undersigned, wish to thank Examiner Landsman for the courtesy and assistance extended on behalf of Applicants during a telephonic interview conducted on January 11, 2005.

In response to the restriction requirement, Applicants have cancelled Claims 4 and 15, without prejudice. Applicants reserve the right to file a divisional application directed to the subject matter of Claims 4 and 15.

The application has been objected to for certain informalities. The Examiner states that the description of Figure 9 does not begin with "Figures 9A-D depict." In response, Applicants have amended the description of Figure 9 to recite "Figures 9A-9D depict." Accordingly, the objection is obviated and withdrawal thereof is respectfully requested.

Claim 5 has been objected to as depending on non-elected claim 4. Claim 16 has been objected to since it depends from claim 5. In response, Applicants have deleted the reference to Claim 4 from Claim 5. Accordingly, the objection to Claims 5 and 16 is obviated and withdrawal thereof is respectfully requested.

Claim 6 has been rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement. The Examiner alleges that the claim does not require the host cell to be

isolated and, therefore, reads on gene therapy.¹ The Examiner recommends that the claim be amended to recite "An isolated host cell."

Applicants have amended Claim 6 in accordance with the Examiner's recommendation. Support for the amendment is found throughout the specification, e.g., on page 17, line 22 to page 18, line 2. Applicants respectfully submit, however, that the specification provides adequate teaching for those skilled in the art to use the subject nucleic acid molecules in a gene therapy setting. Subject matter relating to gene therapy, e.g., as embodied in original Claims 26-37, is canceled in response to the restriction requirement and will be pursued in a divisional application.

Accordingly, the rejection of Claim 6 under 35 U.S.C. § 112, first paragraph, is overcome and withdrawal thereof is therefore respectfully requested.

Claims 14, 16 and 17 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enabling support. In the response to the previous Official Action, Applicants amended Claims 14, 16 and 17 to remove "pharmaceutical" from the preamble, as suggested by the Examiner. However, the Examiner in the present Action alleges that the amended claims are viewed as "reach through" claims. The Examiner alleges that Applicants are attempting to capture methods of treatment which employ these polynucleotides. The Examiner further alleges that Applicants have not provided any guidance or working examples in the specification of the use of the polynucleotides of SEQ ID NO:1 for any therapeutic purpose. Additionally, the Examiner alleges that it would not be predictable to the skilled artisan how to use these polynucleotides for any therapeutic purpose.

¹ The Examiner appears to refer to cells in a host involved in gene therapy.

In response, Applicants respectfully submit that the composition presently claimed can be employed in an *in vitro* setting. Applicants observe that the specification discloses that nucleic acid molecules coding for OGFr can inhibit growth of cells *in vitro* when introduced into the cells. See the specification on page 5, lines 20-24. The specification also teaches that the nucleic acid molecules can be introduced to such cells by well-known procedures, e.g., transfection. See the specification on page 26, line 28 to page 27, line 4. Thus, the specification provides sufficient guidance for one skilled in the art to make and use the claimed compositions of Claims 14 and 16-17, without undue experimentation.

Applicants submit that the specification also adequately teaches how to make and use the claimed composition in a therapeutic method. See page 6, lines 3-24, for example, where the specification discloses a method of treating cancer in a patient by administering the claimed compositions, e.g., an effective amount of a nucleic acid molecule coding for an OGFr.

In addition, Applicants further submit herewith additional data, provided in Exhibit A (a manuscript in press), in support of Applicants' position. Exhibit A describes an experiment conducted by Dr. Zagon, a co-inventor of the present invention, and his colleagues. In the experiment, rat OGFr cDNA (i.e., the sequence as set forth in SEQ ID NO: 1 in the present application) was delivered to the cornea of rats by a Helios Gene Gun system. The result shows that the proliferation of transfected corneal basal cells was only one-third of that in the control cornea. See the Abstract of the manuscript. This result supports the use of the claimed nucleic acid in treating cell proliferation disorders and tumors, as indicated on page 13, last paragraph of Exhibit A, and is consistent with the teaching of the present application.

It is further noted that the Examiner during the telephonic interview indicated that Claims 14 and 16-17 are allowable in their present form.

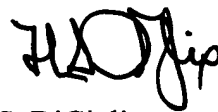
In view of the foregoing, the rejection of Claims 14 and 16-17 under 35 U.S.C. § 112, first paragraph, is overcome and withdrawal thereof is respectfully requested.

Claims 3, 5, 6, 14, 16 and 17 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly introducing new matter. In particular, the Examiner alleges that Claim 3 introduces new matter by reciting a wash step of "about 65°C" and the condition of "about 0.1% SDS." Claims 5-6, 14 and 16-17 depend on Claim 3.

In response, Applicants have amended Claim 3 by reciting the stringent condition as "hybridization at about 42°C and washing at about 60°C in 0.1X SSC with 0.1% SDS." Support for this amendment is found on page 34, lines 2-7, for example. No new matter is introduced. The Examiner indicated during the course of the interview that Claim 3, as amended, is allowable. Accordingly, the rejection of Claims 3, 5, 6, 14, 16 and 17 under 35 U.S.C. §112, first paragraph, is overcome and withdrawal thereof is respectfully requested.

In view of the foregoing amendments and remarks, it is firmly believed that the present application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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Encl.: Exhibit A



**Particle-Mediated Gene Transfer of Opioid Growth Factor Receptor
(OGFr) cDNA Regulates Cell Proliferation
of the Corneal Epithelium**

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Supported in part by NIH grant EY13734.

Structured Abstract:

Purpose. To determine at the molecular level whether interactions between the opioid growth factor (OGF) and OGF receptor (OGFr) play a role in regulating DNA synthesis in the homeostasis of the corneal epithelium. **Methods.** The plasmid pcDNA3.1+OGFr-HA, carrying the rat OGFr cDNA epitope-tagged with a C-terminal hemagglutinin (HA), or the empty-vector (pcDNA3.1+), was delivered twice by the Helios Gene Gun System at 300 psi to the cornea of anesthetized rats. The contralateral (untreated) cornea served as the naive specimen. BrdU was used to determine whether the recombinant OGFr was effective in regulating DNA synthesis in the rat peripheral corneal epithelium. **Results.** Within 18 hr of transfection, positive HA staining was apparent in both the basal and suprabasal layers (efficiency > 90% of the cells) throughout the central and peripheral cornea. Quantitative immunohistochemistry with rhodamine-conjugated anti-OGFr antibodies revealed 2-fold more OGFr expression in the central and peripheral epithelium of transfected corneas relative to naive corneas. The number of BrdU positive basal cells in the peripheral epithelium of the transfected cornea was one-third of that in the naive cornea. **Conclusions.** These data demonstrate the direct role of the OGF-OGFr system in determining cellular renewal in the mammalian corneal epithelium. Moreover, the successful establishment of a novel delivery system of cDNAs to the ocular surface suggests a therapeutic role for gene therapy in the eye.

Key Words: Gene gun - cDNA transfection - BrdU synthesis - Corneal epithelium

INTRODUCTION

The corneal epithelium modulates fluid transport for normal stromal hydration and corneal transparency, and serves as an anatomical and physiological barrier against ocular infection.¹⁻³ This epithelium is in a constant state of renewal, and injury to the epithelium requires prompt resurfacing in order to re-establish visual function.⁴ Although information exists on cellular renewal and repair of ocular surface epithelium,⁵⁻⁷ our knowledge of the molecular processes regulating cell proliferation in the cornea remains incomplete.

The opioid growth factor (OGF) ([Met⁵]-enkephalin), is an endogenous peptide that is autocrine produced⁸ and secreted⁹⁻¹⁶ by basal and suprabasal central and peripheral corneal epithelial cells, as well as limbal and conjunctival cells. OGF interacts with a nuclear-associated receptor, OGFr.¹⁷ OGFr has been cloned and sequenced in humans, rats, and mice.¹⁸ Its nucleotide sequence does not resemble that of classical opioid receptors or the nuclear receptor family, and lacks the motifs of transmembrane proteins.¹⁸ However, OGFr has a characteristic motif related to a nuclear localization signal (NLS),¹⁸ and this receptor is associated with the outer nuclear envelope as detected by immunoelectron microscopy in corneal epithelium.¹⁴ The expression of OGF, and its receptor - OGFr, are conserved in the homeostatic vertebrate cornea,⁹ including humans.^{9,15} Moreover, both OGF and OGFr are expressed in the cornea of diabetic animals,¹⁶ and during wound healing of humans¹⁵ and animals.^{12,13}

The function of the OGF-OGFr system in ocular surface epithelium has been investigated by examining the repercussions of exposure to exogenous OGF during homeostasis,¹¹ wound healing,^{12,13,15} and the outgrowth of explants in tissue culture.¹⁰ The effect of exogenous OGF was to inhibit DNA synthesis, wound healing, and organotypic

outgrowth of ocular surface epithelial cells in a receptor-mediated manner. A second line of investigation has been to utilize opioid-receptor blockade using the potent and long acting opioid-receptor antagonist, NTX.^{10-13,15,16} The results of these studies showed that exposure to NTX increased DNA synthesis, expedited wound healing, and accelerated the outgrowth of explants in culture. The conclusion of these investigations was that the OGF-OGFr system is present in ocular surface epithelium, and its manipulation impacts corneal epithelial cells in culture and during wound healing. Whether OGF is a tonically active inhibitory peptide that transduces its effect directly or indirectly through a receptor-mediated mechanism (presumably OGFr) is unclear.

The study was designed to determine whether the OGF-OGFr axis directly regulates DNA synthesis in homeostasis of the ocular surface epithelium, or if the OGF-OGFr system produces effects on cell replication by way of secondary pathway(s). To address this question, the present study used recombinant DNA technology to overexpress OGFr in the homeostatic corneal epithelium. The outcome of this molecular perturbation on DNA synthesis of basal epithelial cells of the peripheral cornea was determined. The peripheral corneal epithelium was examined because it is a proliferatively active population of cells, in contrast to the central corneal epithelium.¹¹ If the OGF-OGFr system plays a direct role in regulating cell proliferation, then an excess of OGFr would be predicted to increase OGF-OGFr interaction and result in a decrease in the number of basal epithelial cells undergoing DNA synthesis. If there is no change in cell replication levels of basal epithelial cells by addition of OGFr, then it is unlikely that the OGF-OGFr axis is directly involved in regulating cellular events. In order to deliver the OGFr construct to the corneal epithelium, a novel technique - particle-mediated gene transfer (gene gun) - was utilized. This method provides

a rapid, highly efficient, and nontoxic technique for transfection of genes both *in vivo* and *in vitro*,¹⁹⁻²⁵ and allows focal gene delivery. This method does not invoke an immunological reaction. The gene gun has been used successfully for studies of gene delivery to the cornea without ocular damage.^{20-22,24,25}

MATERIALS AND METHODS

Plasmids

The nucleotide sequence for rat OGF α was reported earlier,²⁶ and has been deposited in GenBank under accession number AF 156878. A plasmid pcDNA3.1+OGF α -HA was prepared in the sense orientation (Sense group). The construct contained a C-terminal hemagglutinin (HA) epitope tag (from A/PR/8/34 influenza virus). Both OGF α and HA were under the control of the cytomegalovirus (CMV) immediate early gene enhancer/promoter. An empty vector, pcDNA3.1+, was transfected into some animals (Empty Vector group).

***In Vivo* Gene Transfer by Gene Gun**

Plasmid DNA was purified by Qiagen columns (Chatsworth, CA) and adsorbed onto gold particles (1.6 μ m in diameter) at concentrations of 2 μ g DNA/mg gold particles²⁰ under nitrogen gas according to the procedures recommended by the manufacturer of the Helios Gene Gun System (Bio-Rad, Hercules, CA).

Adult male rats (250-300 g) were obtained from Charles River Laboratories, (Wilmington, MA). All animal experiments conformed to the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research, regulations of the National Institutes of Health, and the Institutional Animal Care and Use Committee guidelines of the Department of Comparative Medicine of The Pennsylvania State University College of Medicine.

Prior to application of the gene gun, rats were anesthetized by i.p. injections of ketamine (50 mg/kg), xylazine (5 mg/kg), and Acepromazine (5 mg/kg). Two drops of 0.5% proparacaine HCl (Alcaine, Bausch & Lomb, Tampa, FL) was applied to the cornea. Only the left eye of each animal was used for experimentation; the right eye served as a naive

(untreated) control. The eyes of all animals were inspected by light microscopy for signs of ocular disease; any rat displaying ocular disease was excluded from the study.

DNA was delivered at a constant distance of 5 cm into a centrally positioned, targeted site of 5 mm diameter on the surface of the cornea using the helium-driven biolistic gene gun. DNA was delivered to the central cornea and a region of the peripheral cornea on each side; the entire rat corneal epithelium is 7-8 mm in diameter.¹¹ Therefore, the corneal scleral limbus and conjunctiva were not treated.

Transfection Efficiency

To test for transfection efficiency, gold particles were monitored in frozen sections of the cornea using brightfield and Nomarski optics; 3 sections/cornea from 3 animals were examined.

Translation of OGFr

Translation of the plasmid over a 48 hr period of time was monitored by staining frozen sections of the rat eye with antibodies to HA (CA5, Roche, Indianapolis, IN). Immunohistochemical staining, using a well-characterized antibody to rat OGFr-fusion protein (I0028),^{14,26} was utilized to identify recombinant OGFr expression. These preparations were quantitated by densitometry using an Olympus BH-2 fluorescent microscope equipped with a PM-10AD digital camera. Sections were monitored with an Olympus UVFL x20 objective (numerical aperture of 0.65) and a digital exposure reading for spot measurement (16 μ m diameter circle). Twenty measurements of the peripheral corneal epithelium were taken randomly from corneas transfected with the sense construct (overexpressing OGFr) or the opposite naive (untreated) cornea. Data were analyzed using paired *t*-tests.

DNA Synthesis

DNA synthesis was measured by injecting rats intravenously with BrdU (100 mg/kg) 2 hr prior to sacrifice. Eyes were removed and prepared for paraffin sectioning. Care was taken that the section traversed through the axial portion of the cornea, bisecting the globe so that the superior/inferior poles were present in the sections.¹¹ Tissue preparations were stained with anti-BrdU-BOD (Roche, Indianapolis, IN) and visualized with stable DAB. The number of BrdU positive cells were counted in the central and peripheral cornea, and in the limbus, from specimens transfected with OGFr (Sense) or empty vector for 18 hr. The opposite naive (untreated) cornea of these transfected subjects also was processed and BrdU labeled cells quantitated. In some cases, rats with transfected corneas (Sense) were injected (i.p.) with either 30 mg/kg NTX or an equivalent volume of sterile saline 4 hr prior to sacrifice; the opposite naive (untreated) cornea from rats receiving sterile saline were included. At least 2 sections/cornea, and 6 corneas/group, were assessed for DNA synthesis. Counting of BrdU positive cells generally followed the procedures for evaluating ³H-thymidine-labeled ocular surface cells reported earlier.¹¹ In brief, using an eyepiece reticule with an 0.1 mm² grid at 400X, a region in the middle of the cornea consisting of 3 grids was monitored as the central cornea. Four grids on each side of the central cornea were counted and considered to be the peripheral cornea. The limbus was assessed in 2 grids at the junction of the cornea and limbus. The central and peripheral cornea had subjacent stroma, whereas blood vessels and non-stromal material were noted underlying the basal epithelial cells of the limbus.

Data Analysis

All BrdU data were analyzed using analysis of variance and Newman-Keuls tests.

RESULTS

Efficiency of Transfections

In preliminary experiments, DNA concentrations (2.0 and 2.5 $\mu\text{g}/\text{mg}$ gold), gold particle size (0.6 and 1.6 μm diameter), and bombardment pressures (100-600 psi) were tested in order to determine optimal conditions that would yield high transfection efficiencies without destroying the morphological integrity of the corneal epithelium.^{27,28} Two bullets of DNA-coated gold particles (DNA concentration, 2.0 $\mu\text{g}/\text{mg}$; 1.6 μm diameter gold) delivered at psi of 300 were utilized. Thus, each rat received approximately 1.6 mg gold. The bullets penetrated all layers of the corneal epithelium (basal and suprabasal), but did not enter the stroma^{27,28} or extend to the limbus or conjunctiva. There was no evidence of corneal damage in any rat from the transfection procedure as judged by corneal clarity, lack of stromal swelling, inflammation, irritation or discomfort at any time throughout the experiments. In addition, the corneal epithelium after transfection appeared comparable to controls by light microscopic examination (Fig.1 at 18 hr, Fig. 2). Under these conditions, transfection efficiency was 90% by counting gold particles in epithelial cells of the peripheral cornea (data not shown).

In Vivo Expression of HA-Tagged Protein and OGF α

A time course for the translation of the OGF α was performed from 6 hr to 36 hr. Immunoreactivity of HA-tagged protein was detected between 16 hr and 27 hr (Fig. 1), with over 90% of the cells immunoreactive with the HA antibody.

OGF α protein in transfected eyes was detected by immunohistochemical staining using a fusion protein antibody specific for OGF α .²⁶ Because of the difficulty in distinguishing the native OGF α from the recombinant OGF α protein, semi-quantitative microscopy was used to calculate light intensity. Thus, to ascertain the relative levels of OGF α immunoreactivity, photodensitometric studies were conducted. Exposure time of the

rat corneal epithelium transfected with sense OGF α cDNA was nearly one-half ($p < 0.001$) that of naive cornea, with exposure readings of 7.2 ± 0.4 and 16.3 ± 0.7 sec, respectively, being recorded (Fig. 2). This increase in immunofluorescence, and hence increased levels of OGF α , were reflected by shorter exposure times.

In Vivo Function of OGF α in the Epithelium of the Ocular Surface

The effects of transfection of OGF α were assessed by analyzing DNA synthesis. To determine that gene gun delivery did not disturb DNA synthesis, corneas transfected with empty vector and naive corneas were compared. The labeling index of BrdU in basal epithelial cells of the empty vector and naive corneas was $11.9 \pm 0.2\%$ and $13.8 \pm 0.2\%$, respectively, with no significant difference recorded.

Examination of basal cells in the peripheral epithelium of corneas transfected with OGF α revealed 58% fewer BrdU-stained cells than in the naive cornea (Figs. 3,4). Corneas transfected with OGF α cDNA and injected with NTX 4 hr prior to sacrifice had a 1.7-fold increase in BrdU labeling of the peripheral epithelium relative to the ocular surface epithelium from naive rat corneas exposed to saline. A 4-fold increase in BrdU labeling of the peripheral corneal epithelium in rats overexpressing OGF α (sense OGF α group) and receiving NTX 4 hr prior to sacrifice was recorded in comparison to corneal epithelium of rats transfected with OGF α cDNA but receiving saline (Fig. 4). The number of BrdU labeled cells in the suprabasal layer of the peripheral cornea did not differ between naive cornea and corneas transfected with OGF α from animals given either saline or NTX (Fig. 4). No differences in the number of BrdU stained cells in the limbus were noted in the naive corneas (7.4 ± 2.1) and corneas transfected with OGF α in the peripheral and central corneal regions from animals given saline or NTX.

CONCLUSIONS

The overall purpose of our studies is to ask whether the OGF-OGFr axis plays an integral role in regulating cell replication of the corneal epithelium, as suggested by indirect evidence gathered in previous experiments with exposure to OGF or to the opioid antagonist, NTX.^{10,11} Our strategy in this investigation was to examine the direct consequences of molecular perturbation of the OGF receptor gene and protein on cell proliferation. Using a particle-mediated gene transfer system (gene gun) to deliver OGFr cDNA that overexpresses the receptor but does not traumatize the cornea, we discovered that DNA synthesis of basal epithelial cells in the peripheral cornea decreased by over half that of values for the naive (untreated) cornea. These data indicate that overexpression of OGFr shifts the balance of the OGF-OGFr axis resulting in down-regulation in terms of cell replicative events, and provides strong evidence that this peptide-receptor system is one of the determinants in cellular renewal of the ocular surface epithelium. The results also suggest that in the homeostatic cornea there is sufficient OGF available to accommodate the increase in OGF receptors made available by transfection. A second line of investigation supporting this conclusion comes from observing the effect of interfering with OGF-OGFr interaction using NTX. DNA synthesis in the basal peripheral epithelial cells of corneas overexpressing OGFr and receiving the powerful opioid receptor blocking agent, NTX, increased dramatically from that of control values, showing that the excess OGFr - like native OGFr - was interacting with OGF. These data support the concept that the OGF-OGFr axis is in a delicate balance and dependent on the quantity of both the receptor and peptide. Thus, overexpression of OGF receptors secondary to gene gun delivery of OGFr changed the kinetics so as provide even more OGFr to interact with OGF, which resulted in

an enhanced reaction (inhibition of DNA synthesis). The molecular based experiments presented herein contribute evidence that the OGF-OGFr axis is i) native to ocular epithelial cells, ii) in an autocrine loop, and iii) a crucial growth regulatory system.

The present study revealed that overexpression of OGFr in suprabasal cells had little effect on levels of DNA synthesis. DNA synthesis in suprabasal cells of corneas overexpressing OGFr, or cells in corneas overexpressing OGFr from animals given NTX, did not differ from values detected in naive corneas. These data support the commonly held belief that suprabasal cells are known to be postmitotic except for displaced basal epithelial cells in the layer proximal to the basal layer.^{29,30} Therefore, it would appear that some processes regulating DNA synthesis - such as the OGF-OGFr axis - are no longer operational in epithelial cells having undergone their final duplication. Perhaps this inability to alter these terminally replicated epithelial cells in the suprabasal layer cells represents a physiologic safety mechanism. This mechanism would serve to prevent a hyperplastic response.

A number of studies have reported that the derivation of the corneal epithelial cells has been linked to stem cells of the limbus.^{31,32} It is interesting to note that we found DNA synthesis in the limbus of corneas transfected with OGFr to be comparable to levels in naive corneas. The area transfected by the gene gun was limited to the corneal epithelium, and did not include the limbus, so it was not expected that the limbal cells would be altered in DNA synthesis. Nevertheless, the inclusion of the limbus for analysis of DNA synthesis served as an internal control, and highlighted the specificity of the transfection procedure and the responses observed. Moreover, this finding that DNA synthesis in the limbus was normal in corneas transfected with OGFr cDNA indicates that overexpression of this

receptor has no cross-communication with the limbus, a region of the cornea that was not included in transfection but serves as a source of cells for this region.

The techniques used in this study for the transfection by the gene gun in rats were modified from the methods described earlier by Tanelian et al.^{21,22} and Shiraishi et al.²⁰ for rabbits. The size of the gold particles, concentration of DNA, and pressure for transfection were comparable between earlier studies and delivery of the cDNA for OGF α , such that it transfected the epithelial layer and did not penetrate the stroma. However, transfection procedures were performed twice in the rats rather than once in the rabbits. Our procedures with the gene gun did not produce trauma of the corneal epithelium, as evidenced by comparable levels of DNA synthesis in transfected and naive corneas. In comparison to other studies on the gene gun and the cornea, which demonstrate successful gene delivery²⁰⁻²² and protein expression,^{20,22} the present study is the first to show the functional ramifications from DNA delivered by particle-mediated gene transfer.

The present study demonstrates that the OGF-OGF α axis directly regulates corneal epithelial cell division in the homeostatic state. Moreover, we establish the potential for the gene gun to introduce functional DNA into the corneal epithelium. In the future, this technique may hold promise for alleviating disorders of corneal epithelial cell division and wound healing. Such disorders might include non-healing diabetic corneal erosions, recurrent erosion syndrome, and even surface epithelial tumors. Further research should be directed at treatments for these clinical conditions.

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Figure Legends

FIG. 1. Temporal course of transfection of the sense-oriented plasmid, pcDNA3.1+OGFr-HA. Rat corneas were transfected using a gene gun and animals killed 6, 18, or 30 hr later. Corneal tissues were processed for immunohistochemistry with an antibody to HA. No immunoreactivity to HA was observed at the 6 hr or 30 hr time points. However, specific staining to HA was detected in the corneal epithelium at 18 hr. Sections stained with secondary antibody only were negative for immunoreactivity (data not shown). ep = epithelium, st = stroma. Bar = 12 μ m.

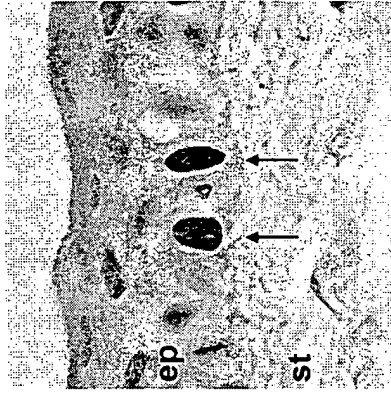
FIG. 2. Immunohistochemical preparations of corneal epithelium from naive eye, or an eye transfected with the sense construct (pcDNA3.1+OGFr-HA), 18 hr after transfection. Tissues were stained with an antibody to OGFr. Note the increase in immunoreactivity in the specimen overexpressing OGFr (Sense) compared to the naive cornea. ep = epithelium, st = stroma. Bar = 12 μ m.

FIG. 3 Preparations of BrdU-labeled epithelial cells (arrows) of the peripheral cornea. Two hr prior to sacrifice, animals were given 100 mg/kg (i.v.) of BrdU. The epithelium of contralateral (untreated) corneas (Naive) in the Sense Saline group served for comparison. Corneas were transfected with pcDNA3.1+OGFr-HA (Sense). Some animals in the Sense group received an injection of 30 mg/kg NTX 4 hr prior to sacrifice (Sense NTX). ep = epithelium, st = stroma. Bar = 9 μ m.

FIG. 4. The number of BrdU-labeled basal epithelial cells in the peripheral corneal epithelium transfected (left eye) with pcDNA3.1+OGFr-HA (Sense) and

examined at 24 hr. Four hr prior to sacrifice, some animals in the Sense group received 30 mg/kg naltrexone (NTX) (Sense NTX) or an equivalent volume of saline (Sense Saline). Two hr prior to sacrifice, these animals were given 100 mg/kg (i.v.) of BrdU. The epithelium of contralateral (untreated) corneas (Naive) in the Sense Saline group served for comparison. Bar = S.E.M. Significantly different from the Naive group at $p < 0.001$ (***). Significantly different number of labeled cells between Sense Saline and Sense NTX groups at $p < 0.001$ (+++).

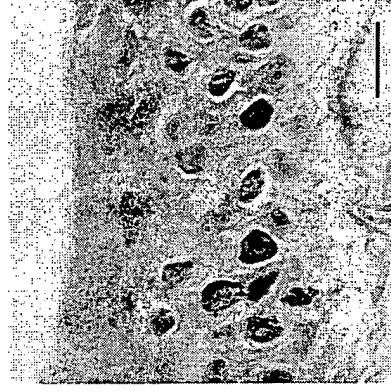
BrdU



Naive

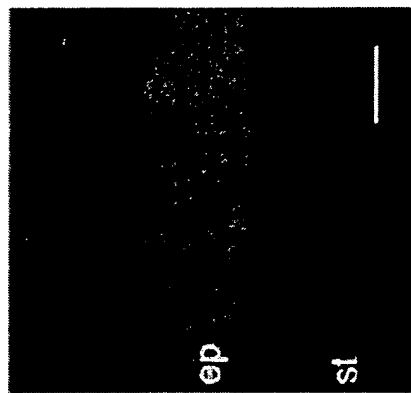


Sense Saline

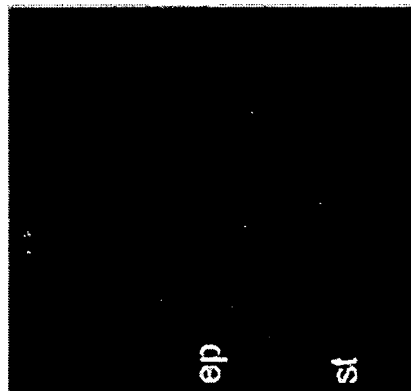


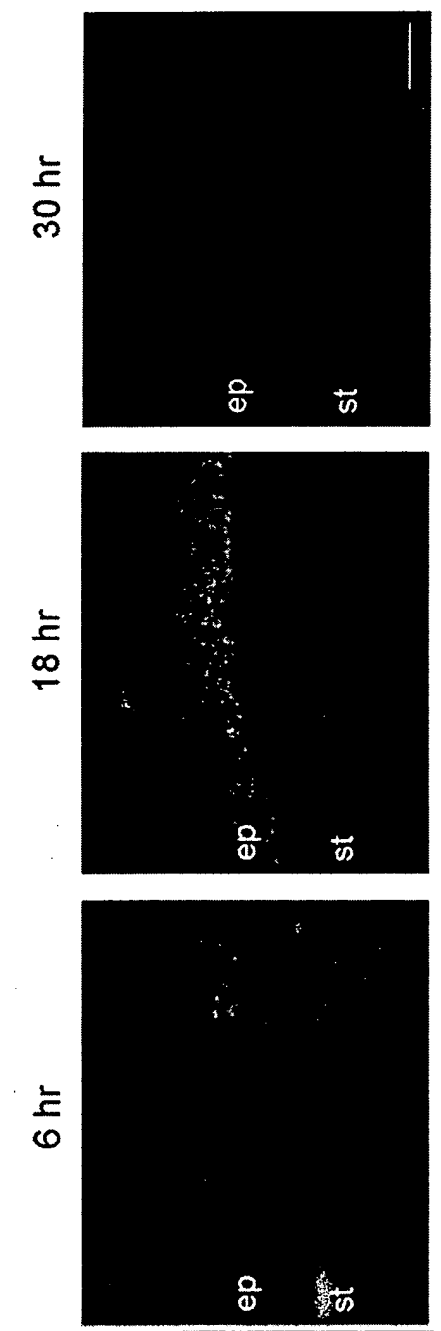
Sense NTX

Sense

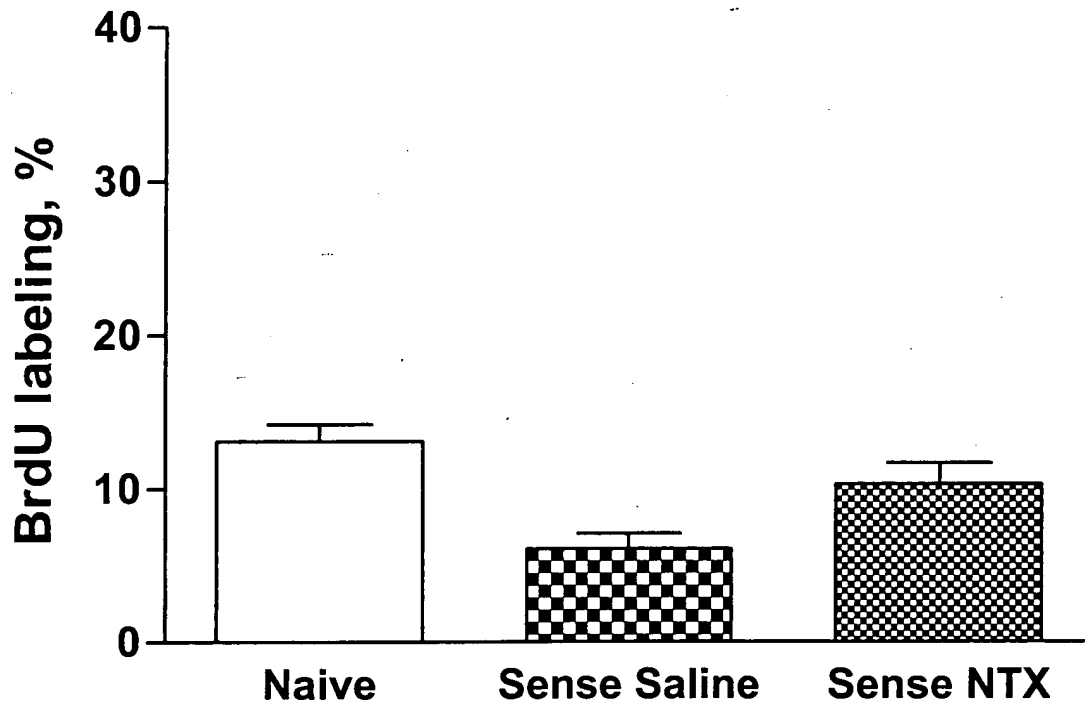


Naïve

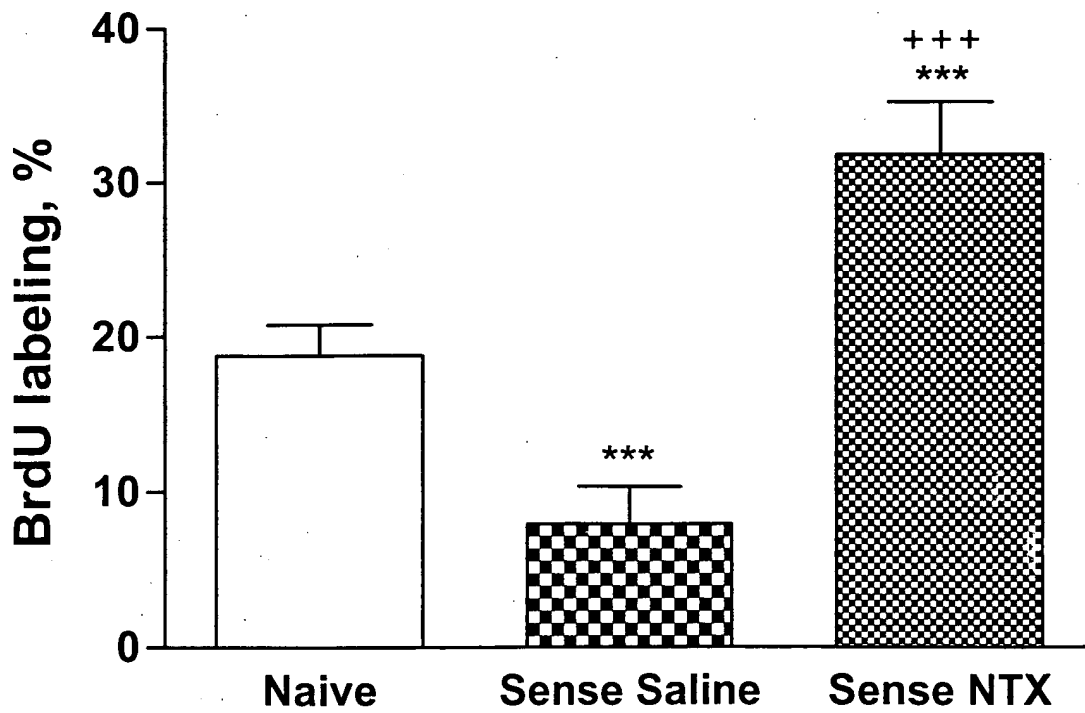




Suprabasal Layer



Basal Layer



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